# New conductive copolymer membranes via track-etched PC templates for biological media ultra-filtration

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# New conductive copolymer membranes *via* track-etched PC templates for biological media ultra-filtration.

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**Abstract**

New microstructurated copolymer membranes have been synthesized using a track-etched polycarbonate (PC) matrix. These membranes proved to be an important device in the field of ultra-filtration and synthetic membranes. These novel structures were obtained by irradiating at various angles (+30°, -30°). Such architecture is expected to improve not only the exchange properties but also, the behaviour under high flow pressure during their use as nanofiltration membranes. Membrane functionalization was performed with an amino acid as a simple biological model. Transmission and ATR FTIR spectroscopies show that the doping state of copolymer dramatically influences the amino acid coupling rate. UV-vis spectroscopy indicates that the copolymer may be self-doped.

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*Keywords:* Polypyrrole, Membrane Irradiation, Track-etched membrane, Surface functionalization, Self-doped polymer.

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## 1. Introduction

Design, fabrication and applications of biochemical sensors have gained considerable importance in recent years. Biosensor is a system consisting of a receptor coupled with a transducer to a detector, thus enabling the conversion of a chemical signal – binding to the receptor – into a physical signal. Conductive polymers, like polypyrrole (PPy), work as transducers [1]. Currently, some applications have been described with immobilized enzymes, DNA, and antibodies into PPy films [1-3]. Immobilization can be carried out with different procedures, mainly by surface adsorption [1]. However, this process suffers from enzyme desorption.

PPy microstructured membranes patterned from track-etched polymer matrix display a smart porous architecture allowing an interesting exchange with two fluids [4, 5]. These membranes proved to be an important device in the field of ultra-filtration and synthetic membranes [6, 7]. Recently, a microstructured membrane based on new PPy derivatives copolymer (poly(Py-*co*-PyCOOH) have been synthesized [4]. This copolymer is of great interest by introducing an anchoring function to create a stable and covalent amide bond between the solid polymer and biological molecules.

In order to combine microstructured membrane and PPy transducer properties, functionalization of PPy derivatives copolymer membranes by biological model molecules is investigated. The aim of this paper is to study molecule covalent binding onto PPy copolymers. A discussion on how conductive polymer doping state may affect the functionalization is also developed.

## 2. Materials and methods

### *2.1 Reagents*

Pyrrole (Py) (Aldrich) was purified by distillation under reduce pressure before use. Pyrrole-3-carboxylic acid (PyCOOH) (Acros) was used as received. Sodium *p*-Toluene sulfonate (*p*TS) and *p*-Toluenesulfonic acid (*p*TSA), *N*-HydroxySuccinimide (NHS), 1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide Hydrochloride (EDC), trifluoroacetic acid (TFA), Triisopropylsilane (TIS), diisopropylethylamine (DIEA), sodium hydroxide, potassium bromide and Ferric Chloride ($FeCl_3$) were purchased from Aldrich, H-Arg(Pmc)-OH from Novabiochem, ethanol from (Fluka) and used as received. Polycarbonate film (PC) was purchased from Goodfellow.

*2.2 Polymer irradiation*

The Swift Heavy Ions (SHI) irradiations were performed using IRRSUD beam line at the GANIL (Caen, France). 9.76 µm thick PC films were irradiated with Pb ions of 0.633 Mev/amu under helium atmosphere with a fluence of $2.10^6$ ions/cm$^2$. 5 irradiations were run on one polymer sample: 4 irradiations at two angles (-30°, +30°) in the (x) and (y) directions and one irradiation, normal to (xy) plane, namely the PC film surface. The total fluence on each PC irradiated film is equal to $10^7$ ions/cm$^2$ ($5x2.10^6$). Track etched PC membranes were obtained by chemical etching of the crossed latent tracks in 2N NaOH at 70°C for 20 min.

*2.3 Preparation of polymer track etched membrane*

Py and PyCOOH copolymerisation onto PC track etched films were performed using the diaphragmatic method described by Zhitariuk *et al*. [5]. The two compartment cell was separated by the PC track etched membrane. One compartment was filled with 10 mL solution (EtOH:$H_2O$) (50:50) of Py (0.15N), PyCOOH (0.15N) and *p*TS (0.1N) as a doping agent. The other one was filled with $FeCl_3$ solution (0.9N) as an oxidant.

Two types of samples were obtained which differ from the presence or not of PC matrix. For the samples without PC matrix, PC template membranes were dissolved in 2N NaOH at 80°C for at least 2h. The obtained black poly(Py-*co*-PyCOOH) membranes were washed three times in ultra pure water. The poly(Py-*co*-PyCOOH) membranes were neutralized with 15% (w/w) $CH_3COOH$ solution for 1h and washed twice with water and dried gently at room temperature.

*2.4 Dedoping method*

Poly(Py-*co*-PyCOOH) membranes were treated with an excess of 4N sodium hydroxide (dedoping process) for 12h at room temperature, then washed with deionised water and dried at 80°C for 2h.

*2.5 Track etched membrane functionalization*

Two methods were used for membrane functionalization depending on whether the track etched template is dissolved or not.

For a sample which contains PC matrix (PPy-Arg-1), functionalization was performed into a two compartment cell. EDC (0.08M) and NHS (0.16M) were dissolved in aqueous medium. The two compartments were filled with 10 mL solution and the mixture was cooled at 4°C. After stirring for 1h at this temperature, the reaction mixture was stirred at room temperature for 3h. The activated membrane was washed twice with distilled water. H-Arg(Pmc)-OH (741 mg, 1.69 mmol) and DIEA(588 µL, 3.38 mmol) were dissolved in aqueous medium and added into the two compartments. The resulting solution was stirred at room temperature for 12h. The membrane was washed in water, dried and a solution of TFA/$H_2O$/TIS (9.5:0.25:0.25) was added to the compartments. After keeping the system for 3h at room temperature, TFA solution was removed and NaOH (2N) was added. After 2h at

80°C, the final membrane without PC matrix was washed again in fresh water and dried gently at room temperature.

For a sample without PC matrix (PPy-Arg-2), membrane was allowed to react with EDC (0.08M) and NHS (0.16M). After stirring 1h at 4°C, the mixture was further stirred at room temperature for 3h. Membrane was washed twice with water. H-Arg(Pmc)-OH (741 mg, 1.69 mmol) and DIEA(588 µL, 3.38 mmol) were dissolved in aqueous medium and membrane was added. After stirring at room temperature for 12h, the resulting membrane was washed twice with water. Afterwards, the membrane was added to a solution of TFA/$H_2O$/TIS (9.5:0.25:0.25). After keeping the system for 3h at room temperature, the final membrane was washed again in fresh water and dried gently at room temperature.

*2.6 Apparatus*

Scanning electron microscopy (SEM) was carried out with a Phillips instrument equipped with a $LaB_6$ tip.

FTIR spectra of polymer membrane powders were recorded on a Nicolet Magma-IR$^{TM}$ 750 spectrometer equipped with a DTGS detector as a KBr pellet. The experiments were carried out by cumulating 32 scans at a resolution of 2 $cm^{-1}$.

ATR-IR spectra were recorded on a Brucker VERTEX 70 spectrophotometer equipped with a PIKE monoreflexion ATR MIRACLE stage. Spectra were obtained by accumulating 32 scans at 4 $cm^{-1}$ at an angle of incidence of 45°.

Ultraviolet-Visible spectra (UV-Vis) were recorded using an UVICAM UV300 spectrometer. Spectra were taken from KBr pellet samples with a KBr window as a reference.

## 3. Results and discussion

*3.1 Polymerisation onto track-etched matrix*

Elaborations of copolymer membranes onto PC microporous membranes template were performed using the diaphragmatic method described by Zhitariuk *et al.* [5]. The copolymerization of the pyrrole and pyrrole-3-carboxylic acid onto PC matrix have been described previously [4].

To introduce new exchange properties for poly(Py-*co*-PyCOOH) membranes, we have chosen to create track-etched polycarbonate films with particular pore orientations. These novel structures were obtained by irradiating at various angles (+30°, -30°) through both X and Y planes (Figure 1). Such architecture is expected to improve not only the exchange properties but also, the behaviour with crushing during their use as nanofiltration membranes.

*3.2 Membranes functionalization*

It is expected that the carboxylic acid groups of the copolymer membranes would allow the anchor molecules of interest by creating an amide bond. Two different synthetic methods are used depending on whether PC membrane template was dissolved (PPy-Arg-2) or not (PPy-Arg-1). Indeed, PC matrix dissolution before or after functionalization may influence the coupling rate. We preliminary choose to couple an amino acid, as simple as arginine, onto the copolymer membrane.

Firstly, the carboxylic acid groups of copolymer membranes were activated by the formation of a succinimide ester catalyzed by a water-soluble carbodiimide, EDC. Afterwards, coupling reaction between the molecule of interest and the activated membrane is allowed to take place (Scheme 1).

After immobilization, lateral chains of arginine were deprotected with a mixture of TFA/$H_2O$/TIS. These procedures were applied to PPy-Arg-1 and PPy-Arg-2.

For PPy-Arg-1, the final step was the dissolution of PC template under basic conditions followed by an acidic neutralization.

*3.3 FT and ATR-IR spectroscopy: polymer and functionalization verification*

Figure 2 displays FTIR spectrum of Poly(Py-*co*-PyCOOH) after polymerisation. Several research groups [8-11] have already assigned vibration modes of PPy. Characteristic absorption peaks are: 1700 $cm^{-1}$ (symmetric C=O stretching vibration of COOH), 1630 $cm^{-1}$ (asymmetric C=O stretching vibration of carboxylate), 1555 $cm^{-1}$ (Py ring stretching vibration), 1476 $cm^{-1}$ (Py ring stretching and C-H in-plane stretching vibration), 1319 $cm^{-1}$ (C-N in-plane deformation), 1201 $cm^{-1}$ (Py ring vibration), 1097 $cm^{-1}$ (asymmetric $S(=O)_2$ stretching vibration), 1049 $cm^{-1}$ (symmetric $S(=O)_2$ stretching and Py ring C-H deformation vibration), 967 $cm^{-1}$ (C-C out of plane ring deformation vibration), 929 $cm^{-1}$ (S-O stretching vibration), 795 $cm^{-1}$ (C-S stretching and $C_\beta H$ out of plane binding), 685 $cm^{-1}$ (C-C out-of-plane ring deformation or C-H rocking).

PC matrix dissolution step proceeds to anion exchange between tosylate and hydroxide (dedoping state). Figure 3A displays FTIR spectrum of Poly(Py-*co*-PyCOOH) after dissolution of PC matrix and acidic neutralization. Several changes in IR spectrum appear in relation with the native copolymer spectrum (Fig.2). Absorption peaks at 1740, 1624 and 1403 $cm^{-1}$ appeared and increased in intensity. These bands can be assigned to the dedoping state of Poly(Py-*co*-PyCOOH). The band which corresponds to Py ring stretching vibration is shifted to 1570 $cm^{-1}$. The band of Py ring deformation (1210 $cm^{-1}$) decreases in intensity. Bands, due to tosylate anion at 1102, 1045, 928 and 792 $cm^{-1}$, diminish significantly.

These results tend to demonstrate that matrix dissolution dedopes partially the polymer. In order to reach a total dedoped state, the polymer was treated with an excess of 4N sodium hydroxide for 12h at room temperature, then washed with deionised water and dried at 80°C

for 2h. After this treatment, the band which corresponds to Py ring stretching vibration is shifted to 1583 $cm^{-1}$ and tosylate bands have disappeared (Fig. 3B).

Consequently, the Poly(Py-*co*-PyCOOH) may exist in different doping states depending on PC matrix dissolution conditions.

After coupling, FTIR spectra of PPy-Arg-1 and PPy-Arg-2 display some variations. FTIR spectrum of PPy-Arg-2 is similar to the FTIR spectrum for (Py-*co*-PyCOOH) after PC matrix dissolution (Fig. 3A). No significant changes were detected. Contrarily, PPy-Arg-1 FTIR spectrum (Fig. 4A) displays four bands associated to arginine guadinine functions at 1772 $cm^{-1}$, 1516 $cm^{-1}$ , 1167 $cm^{-1}$ and 1080 $cm^{-1}$ which correspond to COOH, $N_2C=N$ , C-$NH_2$ and $CH_2$-NH-C groups respectively. The intensity of these bands is in the expected range for a molecule immobilization on a polymer membrane.

It confirms successfull coupling of arginine on the poly(Py-*co*-PyCOOH) membrane. Despite no significant changes were recorded on FTIR spectrum of PPy-Arg-2, immobilization of arginine may have occurred but with a very low yield which is not compatible with the FTIR resolution. A real improvement in functionalization yield is then brought in functionalizing first the copolymer prior to PC matrix removal. It assesses that the dedoping state of the conductive copolymer affects dramatically further functionalizations.
A decrease in tosylate bands intensities (1102, 1045, 928 and 792 $cm^{-1}$) was also noticed on PPy-Arg-1 FTIR spectrum. It is likely that arginine lateral function deprotection which involves the use of trifluoroacetic acid solution participate simultaneously to dedoped partially the conductive copolymer.

FTIR spectra with PPy-Arg-1 and PPy-Arg-2 using Attenuated Total Reflexion mode allow us to determine arginine location in comparison with transmission mode. The same bands at 1772, 1512, 1164 and 1082 $cm^{-1}$ are observed (Fig. 4B) with similar intensities. It

tends to indicate that arginine coupling occurs equally onto copolymer membrane surface and tubules walls.

*3.4 Effect of doping state on coupling rate*

After PC matrix dissolution step, the absorption band of Py ring shifts to higher wavenumbers (1555 to 1583 $cm^{-1}$) which indicates that π electrons are less delocalized [8, 12].

The UV-vis spectra of Poly(Py-*co*-PyCOOH) before and after complete dedoping state are shown in Figure 5. For native copolymer, three absorption peaks at 409 nm, 762 nm and 934 nm were detected. The absorption peak at higher energy is assigned to the $\pi$- $\pi^*$ transition on the basis of polypyrrole systems [13, 14], while that at lower energy are the polaron (762 nm) and bipolaron (934 nm) bands. The dedoped copolymer UV-vis spectrum displays two additional absorption peaks at 470 nm and 668 nm. Band at 470 nm can be assigned to a new $\pi$- $\pi^*$ transition, while absorption peak at 668 nm corresponds to a polaron peak [11]

Several examples of PPy or copolymer which contain sulfonic acid group on the β-position [11] or carboxylic acid group on the N position [15] are well-known to present a self-doping property. If we assume that carboxylate functions of poly(Py-*co*-PyCOOH) play a role as counterions for stabilizing copolymer chain (Scheme 2), it explains the appearance of visible band at 668nm. Indeed, self-acid-doping may generate a polaron peak [11].

This assumption is consolidated by the Py ring peak shift to the higher FTIR wavenumbers as previously mentioned. If β-carboxylate groups play a role as counterions (Scheme 2), their reactivity to create an amide bond slows down.

These results show the importance of synthetic strategy to choose when functionalizing such poly(Py-*co*-PyCOOH) membrane type.

## 4. Conclusion

FT-IR studies of Poly(Py-co-PyCOOH) show that the copolymer membrane presents different doping states. However, we have demonstrated by UV-vis spectroscopy different electronic characteristics which could be assigned to self doping state. Work is in progress in our group to confirm the self-dopping properties of this copolymer. This self doping state can influence dramatically the membrane functionalization rate. It is thus necessary to adapt the synthetic strategies and to privilege immobilization prior to template membrane dissolution.

Moreover, swift heavy ions of very low energy allow us to irradiate PC membranes at various angles. These PC membranes were used as template to Py and PyCOOH oxidative copolymerization. It resulted to novel microstructured copolymer membranes presenting crossed microtubules. Further experiments are planned to evaluate their potentially improved mechanical properties.

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## Figure captions

Scheme 1 : Functionalization of poly(Py-*co*-PyCOOH).

Scheme 2 : Schematic representation of self-doping copolymer.

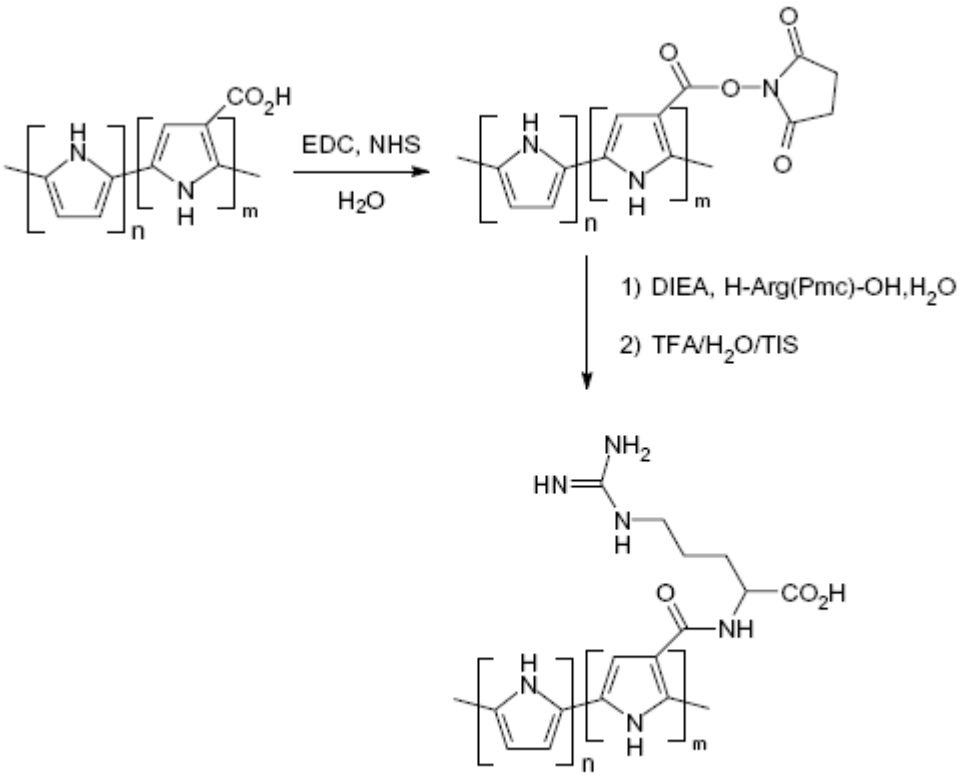
Figure 1 : Microstructured Poly(Py-*co*-PyCOOH) membrane after PC matrix dissolution. Irradiation conditions of initial PC track-etched membrane: Pb ions, (-30°;+30°) (XY), Low energy beam line IRRSUD (GANIL, Caen, France).

Figure 2: FTIR spectrum of Poly(Py-*co*-PyCOOH) after polymerisation.

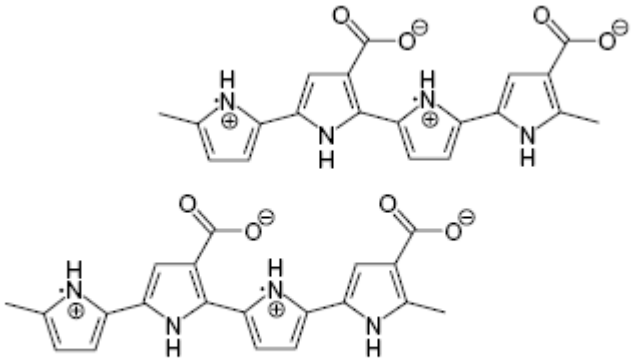
Figure 3: FTIR spectra of Poly(Py-*co*-PyCOOH) A: after PC matrix dissolution. B: after complete dedoping.

Figure 4: FTIR spectra of PPy-Arg-1(dark line) and PPy-Arg-2 (grey line). A: in transmission mode. B: in ATR mode.

Figure 5: UV-vis spectra of (— —) Poly(Py-*co*-PyCOOH) after complete dedoping state and (—) native Poly(Py-*co*-PyCOOH).




Scheme 1. Functionalization of poly(Py-*co*-PyCOOH).



Scheme 2. Schematic representation of self-doping copolymer.

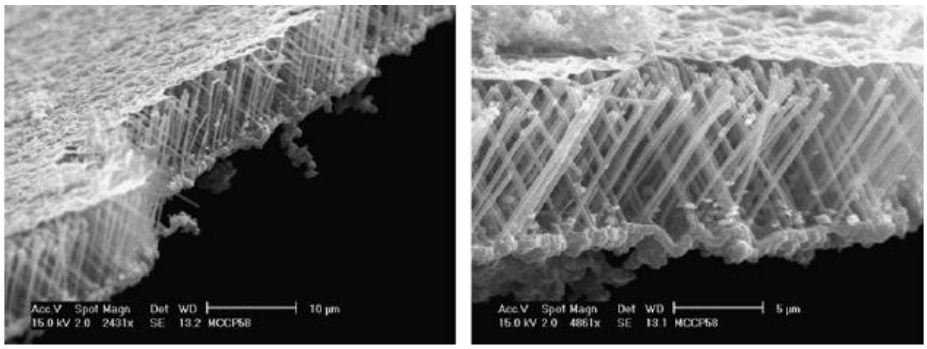


Fig. 1. Microstructured Poly(Py-*co*-PyCOOH) membrane after PC matrix dissolution. Irradiation conditions of initial PC track-etched membrane: Pb ions, (−30°; +30°) (*XY*), Low energy beam line IRRSUD (GANIL, Caen, France).

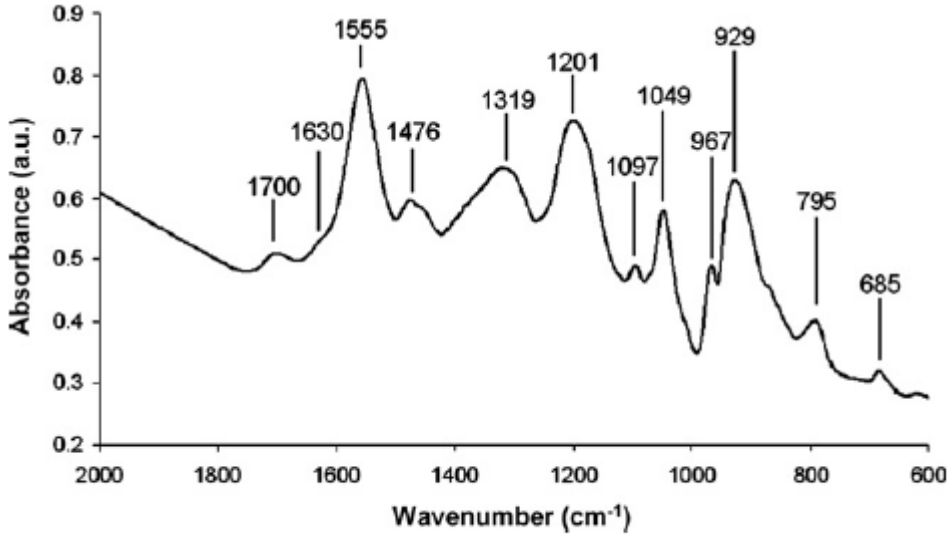



Fig. 2. FTIR spectrum of Poly(Py-*co*-PyCOOH) after polymerisation.

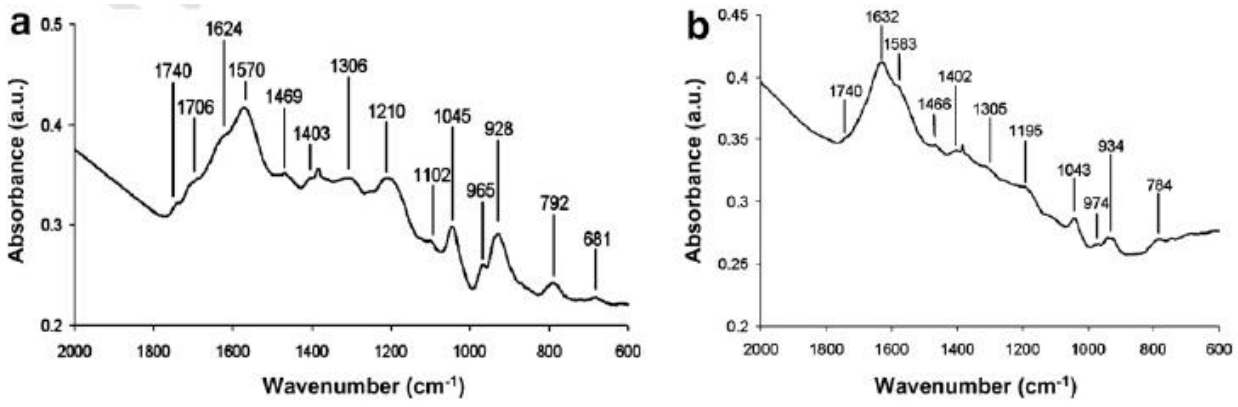



Fig. 3. FTIR spectra of Poly(Py-*co*-PyCOOH) (a) after PC matrix dissolution (b) after complete dedoping.

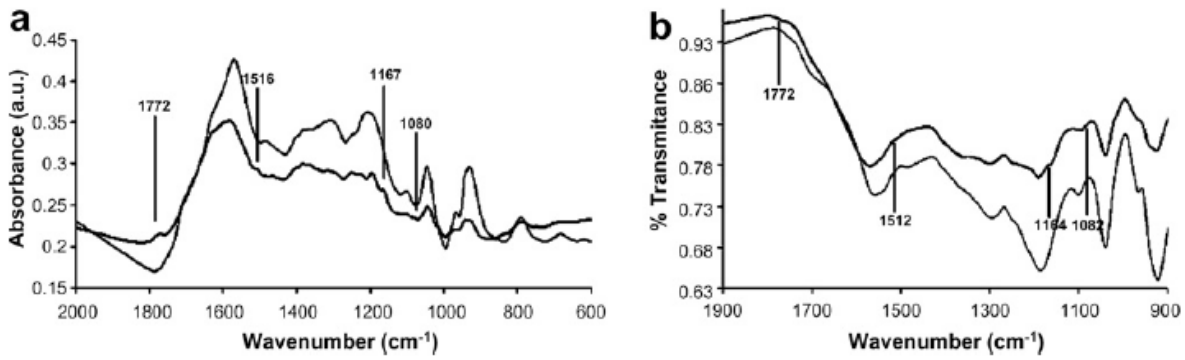



Fig. 4. FTIR spectra of PPy-Arg-1(dark line) and PPy-Arg-2 (grey line) (a) in transmission mode (b) in ATR mode.

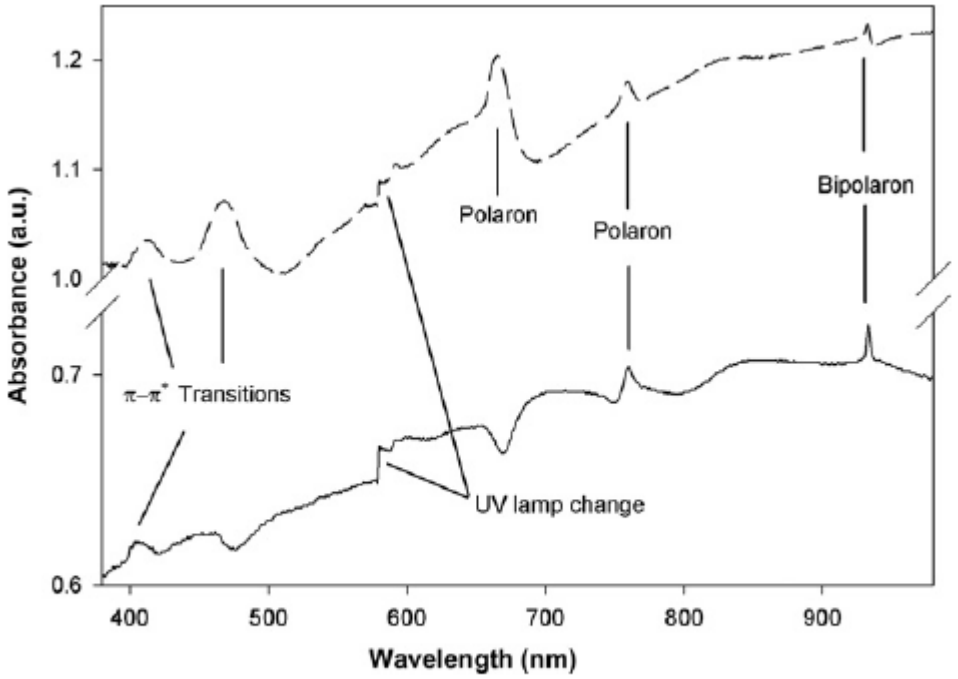



Fig. 5. UV–vis spectra of (——) Poly(Py-*co*-PyCOOH) after complete dedoping state and (—) native Poly(Py-*co*-PyCOOH).